Cerebrovascular Response to Hypoxia in Young vs Aged Rats

WILLIAM E. HOFFMAN, PH.D., RONALD F. ALBRECHT, M.D., AND DAVID J. MILETICH, PH.D.

SUMMARY Cerebrovascular responses of young and aged rats were tested to graded levels of hypoxia using a modification of the Levine ischemic-hypoxic rat model in which one carotid artery was ligated. Rats were anesthetized with 70% N₂O, 30% O₂ and cortical and subcortical cerebral blood flow (CBF) were measured with radioactive microspheres. CBF and cerebral cortical oxygen consumption (CMRO₂) were measured under control conditions and during hypoxia with arterial oxygen content maintained at approximately 9, 5, and 3 ml/dl-1. CBF responses in cortical and subcortical tissues were similar between young and aged under control conditions and during moderate hypoxia (CaO₂ = 9 ml/dl-1). Maximum cerebrovascular responses to severe hypoxia were greater in young than in aged rats and these trends were significant in both ligated and unligated cortical tissue (p < 0.05). CMRO₂ was maintained at control levels during moderate hypoxia but decreased significantly more in aged than in young rats when CaO₂ was decreased to 3 ml/dl-1. These results suggest that baseline CBF and the sensitivity of cerebrovascular receptors to moderate hypoxia are similar in young vs aged rats but that maximum reactivity to severe hypoxia is attenuated in aged subjects. CBF measured after one minute of hypoxia, before the induction of brain tissue acidosis, produced no significant change in the CBF response to hypoxia or in the difference between young and aged rats. Brain tissue pH changes do not appear to be the major factor for mediating CBF increases during hypoxia in young or aged rats, although it may interact with other mediators of the response.

ANESTHETIC MANAGEMENT of the aged patient is often complicated by decreased cardiovascular reserve and preoperative cardiorespiratory disease. This increases the risk of hypoxic-ischemic episodes during anesthetic procedures and produces an increased incidence of postoperative hypoxemia with advancing age. It is important to know whether changes occur in the aged cerebrovasculature may alter the sensitivity or reactivity of these vessels to hypoxic or metabolic stimuli and further increase the risk of hypoxic-ischemic brain damage. However, little is known about these possible changes. Several reports suggest that the cerebral blood flow (CBF) response to hypercapnic and hypocapnic stimuli are attenuated in aged compared to young subjects. On the other hand, Haining et al. reported no difference in the initial CBF response to moderate hypoxia (PaO₂ = 60–70 mm Hg) in young vs aged rats. These results suggest there may be differences in sensitivity and/or reactivity to various cerebral metabolic stimuli in young vs aged subjects. In these experiments we have tested cerebrovascular changes to graded levels of arterial hypoxia and evaluated the possibility that tissue pH changes may mediate these responses. The results indicate that cerebrovascular sensitivity and CBF responses to moderate hypoxic challenges are similar between young and aged rats but reactivity of aged vessels to severe hypoxic challenges is attenuated. Brain tissue pH changes do not play a major role in these responses.

Methods

Male F-344 rats, 6 months and 26–28 months old, were used in these experiments (Charles River Inc.). For surgery rats were anesthetized with halothane, tracheostomized and artificially ventilated with 1% halothane in 70% N₂O and 30% oxygen using a Harvard small animal respirator. PE50 tubing catheters filled with heparinized saline were inserted into both femoral arteries and a femoral vein. A catheter was also inserted into the left ventricle via the right carotid artery. Pressure pulses were monitored using a Hewlett Pack-
ard pressure transducer and strip chart recorder to insure proper placement of the ventricular catheter. Following the completion of this surgery, all incisions were closed with wound clips and the rat placed in a stereotaxic head holder. The skull was exposed, the bone over the sagittal sinus drilled away and a 23 gauge needle inserted into the sagittal sinus stereotaxically. Following the completion of all surgical procedures the halothane was removed from the inspired gases and the rat allowed 30 minutes to stabilize. Arterial PCO2 was adjusted to 35-40 mm Hg. Rectal temperature was measured with a Yellow Springs Inc. thermistor probe and maintained at 37°C using overhead heat lamps. Mean blood pressure was recorded continuously from a femoral artery catheter.

Microspheres

Fifteen micron microspheres, labelled with cobalt-57, ruthenium-103 or scandium-146 (New England Nuclear) were used in these studies. Stock solutions containing 500,000 microspheres/ml were suspended in isotonic saline with 0.01% Tween-80. Ventricular pressure pulses were monitored before each test. Microspheres were vortexed for one minute, 0.2 ml withdrawn (100,000 microspheres), injected into the left ventricle via the ventricular catheter (dead space = 0.06 ml) and flushed in with 0.2 ml saline over 20 seconds. Starting immediately before each microsphere test and continuing 45 seconds after the end of each injection blood was withdrawn from a femoral artery at a rate of 0.4 ml/min using a Harvard infusion-withdrawal pump. Arterial and sagittal sinus blood samples were taken at the end of each test for measurement of blood gases and pH. Using an IL 1303 blood gas analyzer and oxygen content was measured using an IL282 co-oximeter. Oxygen content was calculated as the sum of the co-oximeter value plus 0.03 times the analyzer and oxygen content was measured using an IL 1303 blood gas analyzer. CBF was analyzed according to the methods of Heymann et al.9 CMRO2 was calculated as the average of left and right cortical CBF times arterial-sagittal sinus oxygen content.

Induction of Hypoxia

A modification of Levine’s model of hypoxic-ischemia,10 consisting of unilateral carotid artery occlusion was used in these experiments. Hypoxia was induced by substituting oxygen with nitrogen in the inspired gases. Arterial oxygen content (CaO2) was decreased to approximate levels of 9, 5 and 3.5 ml/dl-1 in both young and aged rats. CO2 was added to the inspired gases during hypoxia to maintain PaCO2 at approximately 35 mm Hg. Hypoxia was maintained for 5-7 minutes before each microsphere test. Microsphere tests were performed randomly in young and aged rats under control and graded hypoxic conditions. Arterial blood pressure was maintained above 100 mm Hg in all tests. For one minute periods of hypoxia, methods were similar to those described above except that one level of hypoxia was induced (CaO2 = 4.2-4.6 ml/dl-1). Microsphere tests were performed 60 seconds after the start of hypoxia. CO2 was not added to the inspired gases in these tests and CMRO2 was not measured.

Data are reported as mean ± SE and statistics performed included unpaired t-tests and two way analysis of variance followed by Duncan’s multiple range test to evaluate specific hypoxic levels when F values were found to be significant.

Results

As shown in table 1, similar levels of graded hypoxia were produced in both young and aged rats. Arterial PaCO2 was maintained at control levels during hypoxia by the addition of CO2 to the inspired gases and arterial blood pressure was maintained above 100

<table>
<thead>
<tr>
<th>Arterial blood pressure (mm Hg)</th>
<th>Heart rate (min-1)</th>
<th>PaO2 (mm Hg)</th>
<th>CaO2 (ml/dl-1)</th>
<th>PaCO2 (mm Hg)</th>
<th>pH</th>
<th>Hemoglobin (g/dl-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young 10</td>
<td>138 ± 7</td>
<td>458 ± 9</td>
<td>121 ± 9</td>
<td>15.1 ± 6</td>
<td>38 ± 1</td>
<td>7.37 ± .02</td>
</tr>
<tr>
<td>13</td>
<td>126 ± 6</td>
<td>452 ± 10</td>
<td>54 ± 4</td>
<td>8.7 ± .3</td>
<td>38 ± 1</td>
<td>7.23 ± .02</td>
</tr>
<tr>
<td>Control 13</td>
<td>130 ± 4</td>
<td>447 ± 12</td>
<td>33 ± 1</td>
<td>5.4 ± .2</td>
<td>38 ± 1</td>
<td>7.26 ± .01</td>
</tr>
<tr>
<td>11</td>
<td>114 ± 6</td>
<td>391 ± 23</td>
<td>25 ± 1</td>
<td>3.3 ± .1</td>
<td>38 ± 1</td>
<td>7.25 ± .01</td>
</tr>
<tr>
<td>Aged 7</td>
<td>136 ± 4</td>
<td>423 ± 21</td>
<td>116 ± 7</td>
<td>17.6 ± 8</td>
<td>36 ± 1</td>
<td>7.40 ± .01</td>
</tr>
<tr>
<td>14</td>
<td>114 ± 5</td>
<td>416 ± 9</td>
<td>41 ± 2</td>
<td>9.0 ± .4</td>
<td>38 ± 1</td>
<td>7.23 ± .02</td>
</tr>
<tr>
<td>9</td>
<td>116 ± 5</td>
<td>370 ± 25</td>
<td>31 ± 2</td>
<td>5.1 ± .3</td>
<td>36 ± 1</td>
<td>7.25 ± .02</td>
</tr>
<tr>
<td>12</td>
<td>113 ± 4</td>
<td>313 ± 17</td>
<td>24 ± 1</td>
<td>3.2 ± .2</td>
<td>38 ± 1</td>
<td>7.22 ± .02</td>
</tr>
</tbody>
</table>

One minute hypoxia

| Young 11                        | 143 ± 4           | 424 ± 14     | 25 ± 1         | 4.2 ± .3      | 27 ± 1 | 7.45 ± .02      | 12.4 ± .5  |
| Aged 9                         | 129 ± 4           | 385 ± 16     | 26 ± 1         | 4.6 ± .7      | 26 ± 1 | 7.44 ± .01      | 13.9 ± .8  |
mm Hg at all hypoxic levels, CBF changes during hypoxia are shown in Table 2. The data are plotted as percent of control in Figure 1. Decreasing arterial oxygen content produced progressive increases in CBF in both young and aged rats. These increases were significantly greater in the left (unligated) cortex and lower brain compared to the right side in both young and aged rats, as analyzed by analysis of variance (p < 0.01), but percentage increases were similar in the right vs the left side (Figure 1). There was no significant difference in the CBF response to hypoxia in subcortical tissue in young vs aged rats, although young rats had a higher maximum CBF response at the most hypoxic level (CaO2 = 3 ml-dl-1). In cortical tissue the CBF response was similar between young and aged during moderate hypoxia. Young rats showed increased CBF at CaO2 values of 5 and 3 ml-dl-1 and this trend was significantly greater compared to aged rats (p < 0.05). CMRO2 changes during hypoxia are shown in Figure 2. CMRO2 was maintained at control levels in both young and aged rats until CaO2 levels of approximately 3 ml-dl-1 were reached. At that point CMRO2 decreased significantly more in aged rats than in young (p < 0.05).

One minute periods of hypoxia performed without the addition of CO2 to the inspired gases produced decreases in PaCO2 and increased arterial pH compared to control values (Table 1). As shown in Table 2, CBF increases during this short period of hypoxia were consistent with changes seen in the first experiment in which arterial pH decreased. In addition, differences in CBF response to hypoxia between young and aged were still apparent.

**Table 2.** Cerebral Blood Flow in Young and Aged F-344 Rats during Hypoxia

<table>
<thead>
<tr>
<th>Arterial oxygen content</th>
<th>Cortex</th>
<th>Lower brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mL O2/100 ml)</td>
<td>Left (mL-100 g-1)</td>
<td>Right (mL-100 g-1)</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15.1±.6</td>
<td>135±10</td>
</tr>
<tr>
<td>13</td>
<td>8.7±.3</td>
<td>247±27</td>
</tr>
<tr>
<td>13</td>
<td>5.4±.2</td>
<td>314±35</td>
</tr>
<tr>
<td>11</td>
<td>3.3±.1</td>
<td>348±23</td>
</tr>
<tr>
<td>Aged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17.6±.8</td>
<td>141±18</td>
</tr>
<tr>
<td>14</td>
<td>9.0±.4</td>
<td>260±20</td>
</tr>
<tr>
<td>9</td>
<td>5.1±.3</td>
<td>299±31</td>
</tr>
<tr>
<td>12</td>
<td>3.2±.2</td>
<td>295±25</td>
</tr>
</tbody>
</table>

Figure 1. CBF changes in young and aged rats during hypoxia. Data are plotted as percent of control flow in each test group. Significance levels indicate difference between test groups at comparable CaO2 value. Results indicate significant difference between young and aged rats which reaches a significant value at severe hypoxic levels in cortical tissue.

Figure 2. CMRO2 changes in young and aged rats during arterial hypoxia. Data indicate maintenance of CMRO2 not significantly different from control levels until severe hypoxic levels were reached (CaO2 = 3.2-3.3 mL-100 ml-1). At that point there was a significantly greater decrease in CMRO2 in aged compared to young rats.
Discussion

The experimental model used in these experiments was originally described by Levine. The premise of this model is that unilateral carotid ligation inhibits the CBF response and adequate oxygenation of the cerebral hemisphere which is ipsilateral to carotid ligation. Hypoxia-ischemia can be produced in the hemisphere ipsilateral to carotid ligation with an hypoxic level which does not significantly depress cardiovascular parameters. This has been verified by Salford and Siesjö and Ginsberg et al, who showed that CBF increases are attenuated in the cerebral hemisphere ipsilateral to carotid ligation compared to the contralateral side. In addition, brain energy state failure occurs in the ipsilateral but not the contralateral hemisphere during hypoxic challenges due to the development of an hypoxic-ischemic state. This model indicates cerebrovascular responses to hypoxia in unligated and collateral (ligated) brain circulation and may have clinical relevance to unilateral internal carotid artery stenosis. The results of these experiments are in agreement with previous reports showing an attenuated increase in CBF in the cerebral hemisphere ipsilateral to carotid ligation during hypoxic challenges. But in terms of percentage increase in CBF, cerebrovascular responses in the left vs the right hemisphere were similar. In both regions, relatively linear increases in CBF were observed, when correlated with changes in CaO2.

This agrees with the work of Traystman et al. The major finding of these studies related to the effect of aging on these cerebrovascular responses. The sensitivity of aged cerebrovascular function to moderate hypoxic challenges is not significantly altered compared to young, but maximal CBF responses during severe hypoxia (CaO2 = 3.2–3.3 ml-dl-1) are attenuated in the aged subjects. This would agree with Haining et al, who found no change in cerebrovascular responses to moderate hypoxia in aged rats, but is also in agreement with other reports describing increased signs of hypoxic damage when the state of hypoxia or ischemia may be severe. Our results would indicate the cerebrovascular response to severe hypoxia is attenuated in aged subjects in cerebral cortical tissue both ipsilateral and contralateral to carotid ligation, indicating an altered response which is not dependent on the collateral circulation.

Because the sagittal sinus blood samples taken in these experiments reflect cerebral cortical drainage from both right and left hemispheres, measured CMRO2 may not reflect changes occurring in the ligated vs the unligated brain hemisphere. This would be true particularly during severe hypoxia in the ligated cortex, when relative ischemia is present and oxygen extraction from arterial blood may be limited. Results of these experiments indicate a stable CMRO2 in both young and aged rats until CaO2 reached levels of 3.2–3.3 ml-dl-1. At that point, significant decreases in CMRO2 were observed in both young and aged rats. The relative stability of CMRO2 until severe hypoxic levels are reached is in agreement with previous authors. In addition, hypoxia (PaO2 = 22 mm Hg) with carotid clamping attenuates the CBF response ipsilateral to the clamp and produces significant decreases in brain energy state in that tissue. Our results indicate that maximal CBF responses to hypoxia are attenuated in aged rats, compared to young, resulting in significantly greater decreases in CMRO2. Further experiments are required to determine if these changes produce greater decreases in brain energy state in aged rats during hypoxia-ischemia.

It has been suggested that the stimulus to increased CBF during hypoxia is a fall in extracellular pH. This is based on several reports, indicating a parallel between extracellular H+ activity and CBF, a correlation between measurable changes in brain tissue lactic acid and CBF during moderate hypoxia, an inverse relation between pial vessel diameter with the pH of the cerebrospinal fluid bathing solution and the inability of hypoxia to produce increases in CBF in hypoglycemic animals in which anaerobic glycolysis and lactic acid production is limited. More recent reports have questioned the pH hypothesis. Siesjö et al reported that increases in CBF are observed during hypoxia induced in rats made severely hypoglycemic, contradicting the previous results of Kogure et al. Other reports indicate lactic acid production following two minutes of hypoxia is insufficient to produce brain tissue acidosis when coincident with a decrease in PaCO2 of 5–8 mm Hg and direct measurement of extracellular pH showed maximal decreases in cerebrovascular resistance after one minute of hypoxia, at a time when tissue pH is unchanged or slightly increased. Results presented here support these previous reports and indicate maximal CBF responses in young and aged rats following one minute of hypoxia in spite of significant decreases in PaCO2 and increases in arterial pH. In addition, the difference in CBF responses to hypoxia was still apparent between young and aged. These data suggest that brain tissue pH changes are not a primary factor mediating cerebrovascular differences between young and aged rats during hypoxia. However, it does not rule out the possibility that tissue pH changes alter cerebrovascular resistance and CBF, particularly during severe hypoxia. The results of Kontos et al indicate that hypoxia dilates cerebral vessels entirely by local mechanisms. Tissue hypoxia may act directly on vascular smooth muscle, inducing relaxation or indirectly by release of vasodilator metabolites from hypoxic cells. More recent reports suggest that brain adenidine may play a major role in the CBF response to hypoxia and ischemia.

In conclusion, results presented here indicate no significant difference in the CBF response to moderate hypoxia between young and aged rats. During severe hypoxia, the increase in CBF was significantly attenuated in the aged rats in cerebral hemispheres both ipsilateral and contralateral to carotid ligation. CMRO2 was also decreased significantly more in aged rats, compared to young, during severe hypoxia, suggesting a greater degree of hypoxic-ischemia in these rats. Further experiments are required to determine how the changes in CBF and CMRO2 may relate to brain ener-
Hypoxia state depletion in young vs aged rats. We found no evidence that brain tissue pH may mediate CBF changes during hypoxia, although the possibility cannot be ruled out that tissue pH may interact with other mediators to influence CBF. The role of other potential mediators of the altered CBF response to hypoxia that occurs with aging remains to be tested.

References

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